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(FILE 'HOME' ENTERED AT 11:44:25 ON 30 SEP 2004)

FILE 'CAPLUS, USPATFULL, MEDLINE, BIOSIS' ENTERED AT 11:44:57 ON 30 SEP  
2004

L1        151433 S G-PROTEIN?  
L2        151433 S G PROTEIN?  
L3        151433 S L1 (L) L2  
L4        61729 S LUCIFERASE  
L5        577 S BRET  
L6        157 S L5 (L) L4  
L7        151433 S L3 (L) L3  
L8        78 S L3 (L) L6  
L9        7745 S GPCR  
L10      51 S L9 (L) L8  
L11      3 S L10 AND PY <2001  
L12      3 S L8 AND PY<2001

L11 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Detection of  $\beta$ 2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET)  
PY 2000  
AU Angers, Stephane; Salahpour, Ali; Joly, Eric; Hilairet, Sandrine; Chelsky, Dan; Dennis, Michael; Bouvier, Michel  
SO Proceedings of the National Academy of Sciences of the United States of America (2000), 97(7), 3684-3689  
CODEN: PNASA6; ISSN: 0027-8424  
AB Heptahelical receptors that interact with heterotrimeric G proteins represent the largest family of proteins involved in signal transduction across biol. membranes. Although these receptors generally were believed to be monomeric entities, a growing body of evidence suggests that they may form functionally relevant dimers. However, a definitive demonstration of the existence of G protein-coupled receptor (GPCR) dimers at the surface of living cells is still lacking. Here, using bioluminescence resonance energy transfer (BRET), as a protein-protein interaction assay in whole cells, we unambiguously demonstrate that the human  $\beta$ 2-adrenergic receptor ( $\beta$ 2AR) forms constitutive homodimers when expressed in HEK-293 cells. Receptor stimulation with the hydrophilic agonist isoproterenol led to an increase in the transfer of energy between  $\beta$ 2AR mols. genetically fused to the BRET donor (Renilla luciferase) and acceptor (green fluorescent protein), resp., indicating that the agonist interacts with receptor dimers at the cell surface. Inhibition of receptor internalization did not prevent agonist-promoted BRET, demonstrating that it did not result from clustering of receptors within endosomes. The notion that receptor dimers exist at the cell surface was confirmed further by the observation that BS3, a cell-impermeable crosslinking agent, increased BRET between  $\beta$ 2AR mols. The selectivity of the constitutive interaction was documented by demonstrating that no BRET occurred between the  $\beta$ 2AR and two other unrelated GPCR. In contrast, the well characterized agonist-dependent interaction between the  $\beta$ 2AR and the regulatory protein  $\beta$ -arrestin could be monitored by BRET. Taken together, the data demonstrate that GPCR exist as functional dimers in vivo and that BRET-based assays can be used to study both constitutive and hormone-promoted selective protein-protein interactions.